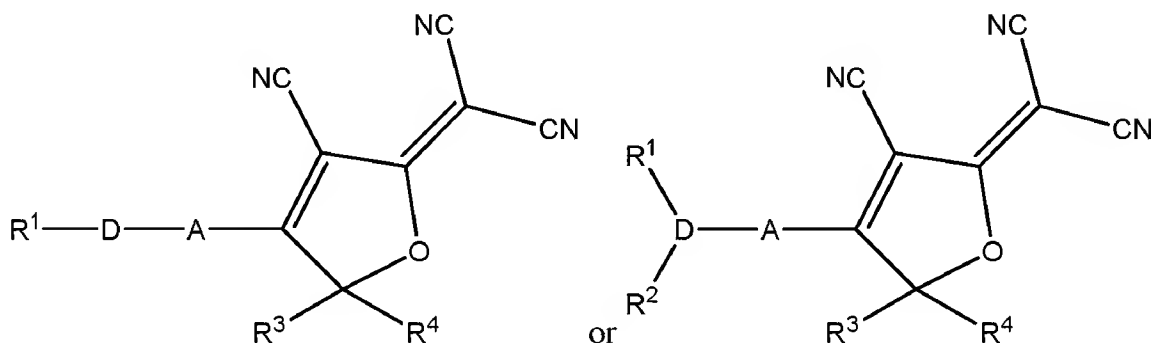


# Claims

- [c1] 1.A composition comprising a fluorophore compound, the fluorophore compound having the chemical structure:



wherein:

D is a donor group having at least one free electron pair conjugated with A;

A is a moiety having at least one multiple bond conjugated with the donor group and the

2-dicyanomethylen-3-cyano-2,5-dihydrofuran group;

R<sup>1</sup> is an alkyl group, alkoxy alkyl group, aromatic group, substituted aromatic group, or hydrogen;

R<sup>2</sup> is an alkyl group, alkoxy alkyl group, aromatic group, substituted aromatic group, or hydrogen;

R<sup>3</sup> is an alkyl group, fluoroalkyl group, aromatic group, or substituted aromatic group;

R<sup>4</sup> is an alkyl group, fluoroalkyl group, aromatic group,

or substituted aromatic group; and the fluorophore compound is not DCDHF-6 (2-[3-Cyano-4-(4-dihexylamino-phenyl)-5,5-dimethyl-5H-furan-2-ylidene]-malononitrile; where A is a benzene ring, D is dihexylamine, R<sup>3</sup> is methyl, and R<sup>4</sup> is methyl).

- [c2] 2. The composition of claim 1, wherein the donor group comprises a nitrogen atom conjugated with A.
- [c3] 3. The composition of claim 1, wherein the donor group comprises an oxygen atom conjugated with A.
- [c4] 4. The composition of claim 1, wherein the donor group comprises a sulfur atom conjugated with A.
- [c5] 5. The composition of claim 1, wherein the donor group comprises a phosphorous atom conjugated with A.
- [c6] 6. The composition of claim 1, wherein A is aromatic.
- [c7] 7. The composition of claim 1, wherein A is benzene, styrene, naphthalene, anthracene, phenanthrene, or pyrene.
- [c8] 8. The composition of claim 1, wherein A is thiophene, furan, pyrrole, imidazole, pyrazole, oxazole, thiazole, diazole, oxadiazole, or thiadiazole.

- [c9] 9. The composition of claim 1, wherein A comprises a vinyl carbon-carbon double bond or an Imine carbon-nitrogen double bond.
- [c10] 10. The composition of claim 1, wherein A comprises a tolane group.
- [c11] 11. The composition of claim 1, wherein the alkyl group is methyl, ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl,  $C_{10}H_{21}$ ,  $C_{12}H_{25}$ ,  $C_{14}H_{29}$ ,  $C_{16}H_{33}$ ,  $C_{18}H_{37}$ ,  $C_{20}H_{41}$ , or  $C_{22}H_{45}$ .
- [c12] 12. The composition of claim 1, wherein the alkoxy alkyl group is methoxymethyl, methoxyethyl, ethoxymethyl, or ethoxyethyl.
- [c13] 13. The composition of claim 1, wherein the fluoroalkyl group is trifluoromethyl or pentafluoroethyl.
- [c14] 14. The composition of claim 1, wherein the compound further comprises at least one functional group suitable for formation of a covalent bond with a biomolecule or biological structure.
- [c15] 15. The composition of claim 14, wherein the functional group is a thiol group, a maleimide group, an iodoacetamide group, an N-hydroxy-succinimide group, a phosphoramidite group, or a methanethiosulfonate

group.

[c16] 16. The composition of claim 14, wherein D comprises the functional group,  $R^1$  comprises the functional group,  $R^2$  comprises the functional group,  $R^3$  comprises the functional group,  $R^4$  comprises the functional group, or A comprises the functional group.

[c17] 17. The composition of claim 1, wherein the compound is DCDHF-MOE  
(2-(4-{4-[Bis-(2-methoxy-ethyl)-amino]-phenyl}-3-cyano-5,5-dimethyl-5H-furan-2-ylidene)-malononitrile),  
DCDHF-1  
(2-[3-Cyano-4-(4-dimethylamino-phenyl)-5,5-dimethyl-5H-furan-2-ylidene]-malononitrile), DCDHF-C6M  
(2-[4-(4-Azepan-1-yl-phenyl)-3-cyano-5,5-dimethyl-5H-furan-2-ylidene]-malononitrile), DCDHF-C5MDM  
(2-{3-Cyano-4-[4-(3,5-dimethyl-piperidin-1-yl)-phenyl]-5,5-dimethyl-5H-furan-2-ylidene}-malononitrile),  
DCDHF-2  
(2-[3-Cyano-4-(4-diethylamino-phenyl)-5,5-dimethyl-5H-furan-2-ylidene]-malononitrile), DCDHF-3  
(2-[3-Cyano-4-(4-dipropylamino-phenyl)-5,5-dimethyl-5H-furan-2-ylidene]-malononitrile), DCDHF-4  
(2-[3-Cyano-4-(4-dibutylamino-phenyl)-5,5-dimethyl-5H-furan-2-ylidene]-malononitrile), DCDHF-5  
(2-[3-Cyano-4-(4-dipentylamino-phenyl)-5,5-dimethyl

-5H-furan-2-ylidene]-malononitrile), DCDHF-8  
(2-[3-Cyano-4-(4-dioctylamino-phenyl)-5,5-dimethyl-5H-furan-2-ylidene]-malononitrile), DCDHF-2EH  
(2-(4-{4-[Bis-(2-ethyl-hexyl)-amino]-phenyl}-3-cyano-5,5-dimethyl-5H-furan-2-ylidene)-malononitrile),  
DCDHF-6-C7M  
(2-[3-Cyano-4-(4-dihexylamino-phenyl)-1-oxa-spiro[4.7]dodec-3-en-2-ylidene]-malononitrile), DCDHF-6-DB  
(2-[5,5-Dibutyl-3-cyano-4-(4-dihexylamino-phenyl)-5H-furan-2-ylidene]-malononitrile), DCDHF-C6M-CF3  
(2-[4-(4-Azepan-1-yl-phenyl)-3-cyano-5-methyl-5-trifluoromethyl-5H-furan-2-ylidene]-malononitrile),  
DCDHF-6-CF3  
(2-[3-Cyano-4-(4-dihexylamino-phenyl)-5-methyl-5-trifluoromethyl-5H-furan-2-ylidene]-malononitrile),  
DCDHF-2-CF3  
(2-[3-Cyano-4-(4-diethylamino-phenyl)-5-methyl-5-trifluoromethyl-5H-furan-2-ylidene]-malononitrile), TH-  
DCDHF-6  
(2-[3-Cyano-4-(5-dihexylamino-thiophen-2-yl)-5,5-dimethyl-5H-furan-2-ylidene]-malononitrile), TH-  
DCDHF-C6M  
(2-[4-(5-Azepan-1-yl-thiophen-2-yl)-3-cyano-5,5-dimethyl-5H-furan-2-ylidene]-malononitrile), TH-  
DCDHF-6-V  
(2-{3-Cyano-4-[2-(5-dihexylamino-thiophen-2-yl)-viny

l]-5,5-dimethyl-5H-furan-2-ylidene}-malononitrile),

DCDHF-2-V

(2-{3-Cyano-4-[2-(4-diethylamino-phenyl)-vinyl]-5,5-dimethyl-5H-furan-2-ylidene}-malononitrile), DCDHF-J-V

(2-{3-Cyano-5,5-dimethyl-4-[2-(2,3,6,7-tetrahydro-1H,5H-pyrido[3,2,1-ij]quinolin-9-yl)-vinyl]-5H-furan-2-ylidene}-malononitrile), DCDHF-6-V

(2-{3-Cyano-4-[2-(4-dihexylamino-phenyl)-vinyl]-5,5-dimethyl-5H-furan-2-ylidene}-malononitrile), DCDHF-2EH-V

(2-[4-(2-{4-[Bis-(2-ethyl-hexyl)-amino]-phenyl}-vinyl)-3-cyano-5,5-dimethyl-5H-furan-2-ylidene]-malononitrile), DCDHF-MOE-V

(2-[4-(2-{4-[Bis-(2-methoxy-ethyl)-amino]-phenyl}-vinyl)-3-cyano-5,5-dimethyl-5H-furan-2-ylidene]-malononitrile), DCDHF-DPH-V

(2-{3-Cyano-4-[2-(4-diphenylamino-phenyl)-vinyl]-5,5-dimethyl-5H-furan-2-ylidene}-malononitrile), DCTA-6C-DCDHF-V

(2-[4-(2-{4-[(6-{4-[Bis-(4-carbazol-9-yl-phenyl)-amino]-phenoxy}-hexyl)-ethyl-amino]-phenyl}-vinyl)-3-cyano-5,5-dimethyl-5H-furan-2-ylidene]-malononitrile), PFP-DDCDHF

(2-{3-Cyano-5,5-dimethyl-4-[1-(4-tridecafluorohexyl-phenyl)-1H-pyridin-4-ylidenemethyl]-5H-furan-2-ylidene}-malononitrile), HP-DDCDHF

(2-{3-Cyano-4-[1-(4-hexyl-phenyl)-1H-pyridin-4-ylidenemethyl]-5,5-dimethyl-5H-furan-2-ylidene}-malononitrile), DOCP-DDCDHF

(4-[4-(4-Cyano-5-dicyanomethylene-2,2-dimethyl-2,5-dihydro-furan-3-ylmethylene)-4H-pyridin-1-yl]-benzoic acid dodecyl ester), P-DDCDHF

(2-[3-Cyano-4-(2,6-dimethyl-1-phenyl-1H-pyridin-4-ylidenemethyl)-5,5-dimethyl-5H-furan-2-ylidene]-malononitrile), 2EHO-DDCDHF

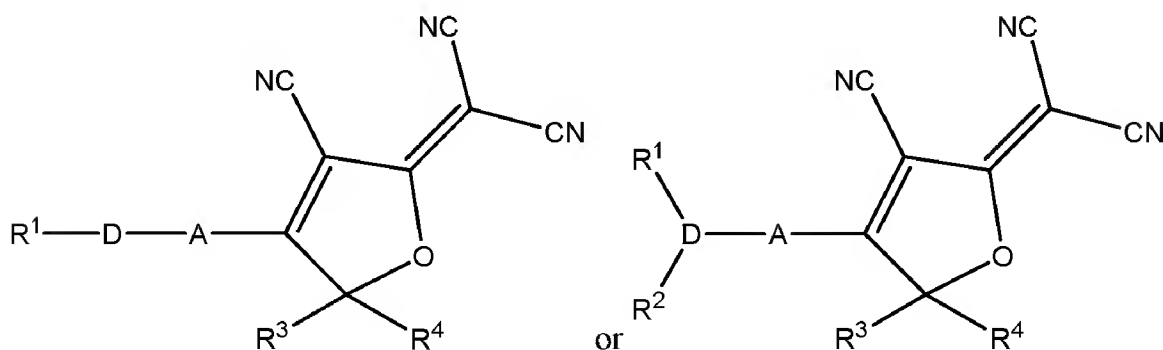
(2-(3-Cyano-4-{1-[4-(2-ethyl-hexyloxy)-phenyl]-2,6-dimethyl-1H-pyridin-4-ylidenemethyl}-5,5-dimethyl-5H-furan-2-ylidene)-malononitrile), M2EHO-DDCDHF

(2-(3-Cyano-4-{1-[3-(2-ethyl-hexyloxy)-phenyl]-2,6-dimethyl-1H-pyridin-4-ylidenemethyl}-5,5-dimethyl-5H-furan-2-ylidene)-malononitrile), or DCDHF-2-2V

(2-{3-Cyano-4-[4-(4-diethylamino-phenyl)-buta-1,3-dienyl]-5,5-dimethyl-5H-furan-2-ylidene}-malononitrile).

- [c18] 18. A method of preparing a fluorescently labeled biomolecule, the method comprising contacting a biomolecule and a fluorophore compound under conditions suitable for bonding of the fluorophore compound

with the biomolecule; wherein the fluorophore compound has the chemical structure:



wherein:

D is a donor group having at least one free electron pair conjugated with A;

A is a moiety having at least one multiple bond conjugated with the donor group and the 2-dicyanomethylen-3-cyano-2,5-dihydrofuran group;

R<sup>1</sup> is an alkyl group, alkoxy alkyl group, aromatic group, substituted aromatic group, or hydrogen;

R<sup>2</sup> is an alkyl group, alkoxy alkyl group, aromatic group, substituted aromatic group, or hydrogen;

R<sup>3</sup> is an alkyl group, fluoroalkyl group, aromatic group, or substituted aromatic group; and

R<sup>4</sup> is an alkyl group, fluoroalkyl group, aromatic group, or substituted aromatic group.

[c19] 19. The method of claim 18, wherein the biomolecule is a nucleic acid.

[c20] 20. The method of claim 18, wherein the biomolecule is



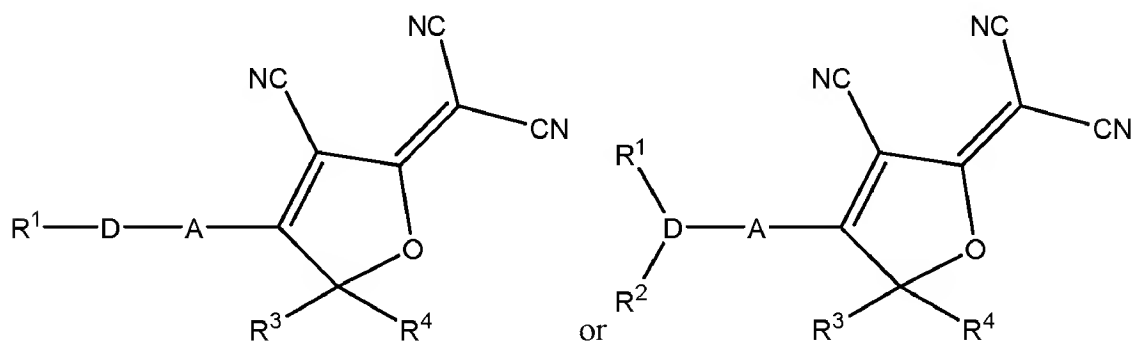
a protein.

- [c21] 21. The method of claim 18, wherein the biomolecule is a peptide.
- [c22] 22. The method of claim 18, wherein the biomolecule is a monosaccharide or a polysaccharide.
- [c23] 23. The method of claim 18, wherein the biomolecule is a nucleotide.
- [c24] 24. The method of claim 18, wherein the biomolecule is a lipid.
- [c25] 25. The method of claim 18, wherein the bonding comprises formation of a covalent bond.
- [c26] 26. The method of claim 18, wherein the bonding comprises formation of an ionic bond, a pi-pi stacking interaction, a hydrophobic interaction, or van der Waals interaction.
- [c27] 27. The method of claim 18, wherein the compound further comprises at least one functional group suitable for formation of a covalent bond with a biomolecule or biological structure.
- [c28] 28. The method of claim 27, wherein the functional group is a thiol group, a maleimide group, an iodoac-

etamide group, an N-hydroxy-succinimide group, a phosphoramidite group, or a methanethiosulfonate group.

- [c29] 29. The method of claim 27, wherein D comprises the functional group,  $R^1$  comprises the functional group,  $R^2$  comprises the functional group,  $R^3$  comprises the functional group,  $R^4$  comprises the functional group, or A comprises the functional group.
- [c30] 30. The method of claim 18, further comprising a step of detecting the biomolecule after the contacting step.
- [c31] 31. The method of claim 18, further comprising a step of analyzing the fluorescently labeled biomolecule, the analyzing step selected from the group consisting of detecting fluorescence, detecting polarization, detecting anisotropy, detecting fluorescence lifetime, detecting spectrum, determining correlations, and detecting second harmonic.
- [c32] 32. A method of preparing a fluorescently labeled biological structure within a cell, the method comprising: providing a cell or cells comprising a biological structure; and contacting the cell or cells with a fluorophore compound under conditions suitable for cellular uptake of the fluo-

rophore compound and bonding of the fluorophore compound with the biological structure; wherein the fluorophore compound has the chemical structure:



wherein:

D is a donor group having at least one free electron pair conjugated with A;

A is a moiety having at least one multiple bond conjugated with the donor group and the

2-dicyanomethylen-3-cyano-2,5-dihydrofuran group;

R<sup>1</sup> is an alkyl group, alkoxy alkyl group, aromatic group, substituted aromatic group, or hydrogen;

R<sup>2</sup> is an alkyl group, alkoxy alkyl group, aromatic group, substituted aromatic group, or hydrogen;

R<sup>3</sup> is an alkyl group, fluoroalkyl group, aromatic group, or substituted aromatic group; and

R<sup>4</sup> is an alkyl group, fluoroalkyl group, aromatic group, or substituted aromatic group.

[c33] 33. The method of claim 32, wherein the biological structure is a lipid bilayer, a membrane, a micelle, the

cytoskeleton, a nucleosome, a ribosome, a peroxisome, a liposome, a plastid, a transmembrane protein, a chloroplast, or a mitochondrion.

- [c34] 34. The method of claim 32, wherein the bonding comprises formation of a covalent bond.
- [c35] 35. The method of claim 32, wherein the bonding comprises formation of an ionic bond, a pi-pi stacking interaction, a hydrophobic interaction, or van der Waals interaction.
- [c36] 36. The method of claim 32, wherein the compound further comprises at least one functional group suitable for formation of a covalent bond with a biomolecule or biological structure.
- [c37] 37. The method of claim 32, wherein the functional group is a thiol group, a maleimide group, an iodoacetamide group, an N-hydroxy-succinimide group, a phosphoramidite group, or a methanethiosulfonate group.
- [c38] 38. The method of claim 32, wherein D comprises the functional group,  $R^1$  comprises the functional group,  $R^2$  comprises the functional group,  $R^3$  comprises the functional group,  $R^4$  comprises the functional group, or A comprises the functional group.

[c39] 39. The method of claim 32, further comprising a step of detecting the biological structure after the contacting step.

[c40] 40. The method of claim 32, further comprising a step of analyzing the fluorescently labeled biological structure, the analyzing step selected from the group consisting of detecting fluorescence, detecting polarization, detecting anisotropy, detecting fluorescence lifetime, detecting spectrum, determining correlations, and detecting second harmonic.